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A LABORATORY BIOASSAY FOR MONITORING RESISTANCE IN TARNISHED PLANT BUG POPULATIONS TO NEONICOTINOID INSECTICIDES

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Abstract

A laboratory bioassay was developed for testing tarnished plant bug populations for resistance development to the neonicotinoid insecticides imidacloprid and thiamethoxam. The bioassay allows for the determination of LC_{50} values by feeding known doses of the insecticides to adult tarnished plant bugs in a honey-water solution. Field populations of plant bugs from several locations in the mid-South were tested with the oral bioassay for resistance to imidacloprid in 2005 and 2006. Resistance to imidacloprid increased 2.46-fold on average in 2006 as compared to 2005. Thiamethoxam was first used in the oral bioassay to test field populations of plant bugs in 2006. The data collected in both years with both insecticides will provide a basis for comparison to determine changes in resistance in future resistance monitoring studies.

Introduction

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is an important pest of cotton grown in the mid-South that is controlled in cotton exclusively with insecticides. Plant bugs have developed resistance to pyrethroid insecticides in many parts of the mid-South (Pankey et al. 1996, Hollingsworth et al. 1997, Snodgrass and Scott 2000). Populations have also been found that have high levels of resistance to several organophosphate and carbamate insecticides including acephate, dicrotophos, malathion, and oxamyl (Zhu et al. 2004, Snodgrass 2006). No resistance to the neonicotinoid insecticides Centric® (thiamethoxam) and Trimax® (imidacloprid) has been reported. Both of these relatively new insecticides are systemic in cotton plants and are used to control sucking insect pests of cotton. Imidacloprid has little contact activity for plant bugs, while thiamethoxam is active against plant bug by contact and ingestion. Both insecticides have good activity against plant bugs in field tests in the mid-South (Ngo and Mascarenhas 2002, Greene and Capps 2003, Layton et al. 2003, Walsh et al. 2003).

Because of resistance to pyrethroid, organophosphate, and carbamate insecticides that can occur in plant bug populations in the mid-South, the neonicitinoid insecticides are very important in resistance management in this area. These insecticides provide a different class of insecticides to which no resistance has developed that can be alternated with other insecticide classes to try and delay resistance development to them. Typical laboratory bioassays for resistance monitoring which rely on contact activity will not work with imidacloprid because it must be ingested. A new bioassay was developed and used for determining resistance levels in plant bug populations to imidacloprid. Since thiamethoxam is also active against plant bugs when ingested, the bioassay was also used to determine resistance levels to thiamethoxam. Plant bug populations were also tested for resistance to thiamethoxam using a contact bioassay. The bioassays and results obtained by testing field populations with them are described in this manuscript.

Materials and Methods

The bioassays were conducted using 20-ml glass liquid scintillation vials. In the oral bioassays (with imidacloprid and thiamethoxam), a piece of floral foam (Oasis Floral Products, Kent, OH) approximately 12-mm in diameter and 12-mm in depth was placed in each vial. The pieces were cut from blocks of floral foam using a number 10 cork borer. This sized piece of floral foam would hold 0.5 ml of fluid without leaking, and Teague and Tugwell (1996) found that plant bugs would feed on fluids placed in the floral foam. Honey-water solution (0.5 ml) was pipetted onto the floral foam piece in each vial and then a single adult was placed in each vial for testing. This amount of honey-water solution would keep a single adult plant bug alive for 72 h without adding more solution. The honey-water solution was 10% by weight. Known doses of imidacloprid or thiamethoxam were fed to plant bugs by

mixing them in the honey-water. Each dose was delivered in the 0.5 ml of honey-water placed in the floral foam piece in each vial. The imidacloprid was technical grade and was purchased from Chem Service, West Chester, PA. Technical grade thiamethoxam was obtained from Syngenta (Greensboro, NC). After a plant bug was placed in a vial, a cotton ball was placed in the opening to confine the plant bug. At least five different doses were used with each test population, and the doses in each test were replicated four times. Ten vials (1 adult per vial) were used in each replication. Control vials received only the honey-water solution, and control mortality was rare and never > 10%. Mortality was determined after 72 h in the oral bioassay using imidacloprid, and after 24 h when thiamethoxam was used. The methods used to conduct the contact bioassay with thiamethoxam are found in Snodgrass (1996). In the contact bioassay, at least five doses were also used with each test population, and the doses were replicated three times. Five vials (2 adults per vial) were used in each replication. Control vials were treated only with acetone, and mortality was low and never exceeded 10%. Tests were conducted at room temperature and humidity was not controlled. Data were corrected for control mortality using Abbott's (1925) formula. Data from the bioassays were analyzed assuming the probit model (Proc Probit: SAS Institute 1999). LC_{50} values for each location were compared to the LC_{50} values for imidacloprid or thiamethoxam for susceptible bugs to obtain a resistance ratio (RR). Differences in LC_{50} values were considered significant if the 95% CL of the resistance ratio at the LC_{50} level did not include 1.0 (Robertson and Priesler 1992).

Test populations of plant bugs were collected for testing with imidacloprid from weeds near cotton fields in 2005 and 2006 in May through September in each year. The same collection locations were used 13 times in both years (Table 1). Adults were collected with a sweep net and held in the laboratory on green bean pods for 24 h before they were used in a test. The susceptible bugs were collected from weeds near Crossett, AR where no row crops are grown. Bioassays with thiamethoxam were conducted in Sepember and October 2006 using plant bug populations collected from nine locations (Table 2).

Results and Discussion

Both imidacloprid and thiamethoxam were active at similar doses in the oral bioassays. The mean LC_{50} for imidacloprid for the test populations was 1.10 and 2.70 µg in 2005 and 2006, respectively (Table 1). The mean LC_{50} for thiamethoxam was 1.50 µg (Table 2). However, thiamethoxam was faster acting than imidacloprid and mortality was determined in the bioassays after 24 h with thiamethoxam as compared to a 72 h exposure period for imidacloprid.

Only 4 populations (Indianola, Clarksdale, Rolling Fork, and Tunica) of the 15 populations tested for resistance to imidacloprid in 2005 had LC_{50} values significantly higher than the LC_{50} value found for susceptible plant bugs from Crossett (Table 1). The average LC_{50} value for the 15 populations tested was 1.10 µg of imidacloprid as compared to a LC_{50} of 0.85 µg for the susceptible population from Crossett. All but one (Parkdale) of the populations tested with imidacloprid in the oral bioassay in 2006 had LC_{50} values significantly higher than the LC_{50} value for the susceptible Crossett population. Nine of the 13 populations tested at the same location in both years had significantly higher LC_{50} values in 2006 as compared to 2005. The highest LC_{50} value was 6.48 µg of imidacloprid (a resistance ratio of 7.6) for the population tested from Clarksdale. The average LC_{50} for imidacloprid for the 19 locations in 2006 was 2.70 µg which was 2.46-fold higher than in 2005. It is not known how the increase in resistance found in 2006 will affect the efficacy of imidacloprid for plant bugs. Resistance monitoring for imidacloprid will be conducted again in 2007. As many populations as possible found with elevated resistance to imidacloprid in 2007 will be tested in the field to try and determine any effect of the resistance on control efficacy.

In the oral bioassays conducted with thiamethoxam, none of the LC_{50} values obtained for the 9 test populations were significantly higher than the LC_{50} value found for the susceptible plant bugs from Crossett (Table 2). The results from the contact bioassays were more variable and 5 of the 9 populations tested had LC_{50} values significantly higher than the LC_{50} value found for susceptible plant bugs from Crossett. To determine if resistance to thiamethoxam is changing, resistance monitoring will be conducted in the same locations in 2007. The oral bioassay developed in this study will be a very useful method for monitoring resistance development to imidacloprid and thiamethoxam. The data presented in this manuscript also provides baseline data for comparison of changes in resistance.

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Table 1. Results from testing tarnished plant bug adults from different locations in the mid-South with imidacloprid. Plant bugs were fed different doses of imidacloprid in honey-water, and mortality was determined at 72 h.

Location	LC_{50}[a] (2005)	RR	LC_{50} (2006)	RR	Change in LC_{50} (2005 to 2006)
Crossett, AR	0.85	----	-----	----	------
Indianola, MS	2.16	2.5	2.24	2.6	+ 0.1
Stoneville, MS	1.10	1.3			
Hollandale, MS	0.98	1.2	5.13*[c]	6.0	+ 4.2
Clarksdale, MS	1.89	2.2	6.48*	7.6	+ 4.6
Mound Bayou, MS	0.64	0.8	1.96	2.3	+ 1.3
Rolling Fork, MS	1.87	2.2	2.74	3.2	+ 0.9
Merigold, MS	1.18	1.4	4.11*	4.8	+ 2.9
Greenville, MS	0.81	1.0	1.48	1.7	+ 1.0
Tunica, MS	1.42	1.7	2.66*	3.1	+ 1.2
Drew, MS	0.64	0.8	3.34*	3.9	+ 2.7
Lambert, MS	0.45	0.5	1.92*	2.3	+ 1.5
Leland, MS	1.34	1.6			
Oak Grove, LA	0.99	1.2	1.73*	2.0	+ 0.7
McGhee, AR	0.95	1.1	2.51*	3.0	+ 1.6
Lake Village, AR	0.55	0.7	3.48*	4.1	+ 2.9
Marks, MS			2.97	3.5	
Ruleville, MS			1.46	1.7	
Avon, MS			2.05	2.4	
Minter City, MS			1.79	2.1	
Parkdale, AR			1.10	1.3	
Vance, MS			3.19	3.8	
Mean	1.10		2.70		+1.9

a. LC_{50} values are in micrograms of imidacloprid.
b. The plant bugs from Crossett, AR were the susceptible population. RR (resistance ratio) was calculated by dividing the LC_{50} value for a location by the LC_{50} value for Crossett.
c. * indicates that the LC_{50} of the population in 2006 was significantly higher than the LC_{50} of the population from the same location in 2005.

Table 2. Results from testing tarnished plant bugs collected from different locations in the mid-South for resistance to thiamethoxam. Plant bugs were tested in glass-vial bioassays by contact activity and by oral ingestion of thiamethoxam in honey-water. Mortality was determined at 24 h.

Location	LC_{50}[a] (contact)	RR[b]	LC_{50} (oral)	RR
Crossett, AR	5.8	---	1.4	---
Vance, MS	33.9*[c]	5.8	1.7	1.2
Rolling Fork, MS	18.2*	3.1	1.6	1.1
Leland, MS	16.8 *	2.9	2.2	1.5
Clarksdale, MS	9.5	1.6	0.6	0.4
Merigold, MS	9.0	1.6	1.1	0.8
Hollandale, MS	12.8*	2.2	2.3	1.6
Winterville, MS	19.6*	3.4	1.7	1.2
Avon, MS	5.2	0.9	0.4	0.2
Indianola, MS	8.6	1.5	1.8	1.2
Mean	14.9	2.6	1.5	1.0

a. LC_{50} values are in micrograms of thiomethoxam.
b. The population from Crossett, AR was the susceptible population. RR (resistance ratio) was calculated by dividing the contact or oral LC_{50} value from a location by the contact or oral LC_{50} value from the Crossett population.
c. * indicates that the LC_{50} of the population was significantly higher than the LC_{50} for the susceptible population from Crossett, AR.